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Polychlorinated Biphenyls Cause Developmental Enamel Defects in Children

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Key Words
Children · Enamel defects · Polychlorinated biphenyls

Abstract
The aim of this study was to evaluate the effects of long-term exposure to polychlorinated biphenyls (PCBs) on developing dental enamel. 202 8- to 14-year-old children who were pre- and post-natally exposed to PCBs in the contaminated region of Bela Krajina, Slovenia, were studied. 202 controls from Brščljin were matched for age and sex. Risk assessment was based on the concentrations of toxic PCB congeners in the diet. Levels of PCBs in dentine were used to validate exposure. PCB levels were analysed by high-resolution gas chromatography. The prevalence of developmental defects of enamel was assessed using the FDI Index. Developmental defects of enamel in permanent teeth were found in 71.3% of exposed children, compared to 49.5% in the control group. The enamel was abnormal in 21.9% of the permanent index teeth of exposed children and in 12.7% of the control children. The difference was statistically significant ($\chi^2 = 84.18$; $p = 0.0019$), mostly on account of demarcated opacities and hypoplasia. The extent of the defects was also greater in the exposed group ($\chi^2 = 61.3$; $p = 0.0001$). No significant correlations were found between PCB exposure and developmental defects in deciduous teeth. In conclusion, our results showed that long-term exposure to PCBs may cause developmental defects of enamel.

Developing enamel is sensitive to a wide range of local and systemic disturbances [Small and Murray, 1978]. Because of the absolute metabolic stability of its structure, changes in enamel during its development are permanent in nature. The prevalence of developmental defects of enamel appears to have increased in recent years. Whilst it may not be the sole cause of this rise, exposure to environmental pollutants has been suggested to have contributed to the problem [Kierdorf et al., 1993; Alaluusua et al., 1996b; Brook et al., 1997].

Polychlorinated biphenyls (PCBs) are persistent polyhalogenated aromatic hydrocarbons that are widespread environmental contaminants. Being lipophilic, PCBs increase in concentration up the food chain and accumulate in human tissues [Safe, 1994]. In the general population, predominant exposure to PCBs is via ingestion of food. PCBs also cross the placenta and are excreted in milk [Ahlborg et al., 1992].

Animal studies in several species have found that PCB exposure can lead to severe morphological changes in ameloblasts [Hashiguchi et al., 1985; McNulty, 1985]. There is strong evidence suggesting a common mechanism of action and also a number of common toxic responses similar to those observed for tetra-chloro-dibenzo-p-dioxin (TCDD) [Safe, 1994]. Partanen et al. [1998] have shown that mouse embryonic teeth are affected in culture by TCDD, depending on epidermal growth factor receptor expression.

The specific risk to humans from PCB exposure is unclear. In two episodes of epidemic PCB poisoning in Asia, excess of ectodermal defects and developmental delay [Ahlborg et al., 1992], including a variety of dental changes

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such as mottled, chipped and carious teeth, have been reported [Hara, 1985; Rogan et al., 1988]. Nevertheless, cocontamination with polychlorinated dibenzo-furans (PCDFs) was largely responsible for the overall toxicity [Safe, 1994; Masuda, 1996]. A recent study found that developmental dental defects were correlated with the total exposure to polychlorinated aromatic hydrocarbons via mother's milk [Alaluusua et al., 1996a], suggesting that developing human teeth are vulnerable to these compounds. The correlation was strong with exposure to prevailing levels of polychlorinated dibenzo-p-dioxins (PCDD) and furans (PCDF) but weak with exposure to PCBs alone [Alaluusua et al., 1999]. Further investigation is needed to determine if PCBs at high levels alone cause developmental impairment in humans.

In the Bela Krajina region of Slovenia, in the 1970s, PCBs from an electro-industrial plant and its waste deposit tips contaminated the surrounding agricultural area. In the plant, technical PCB mixtures (mostly Pyralene-1500 and -3000) were used, with negligible amounts of toxic PCDFs [Hong et al., 1993]. This provided an opportunity to evaluate the risk of PCBs alone in humans. Numerous interdisciplinary studies were conducted during the years following the accident [Jan and Adamič, 1991; Zupančič-Kralj et al., 1992]. There were no major short-term health effects, although PCBs in human tissues reached a level two orders of magnitude greater than the background level in Slovenia [Tretjak et al., 1991].

Due to their widespread distribution, persistence, bioaccumulation and toxicity in animal studies, the potential impact of PCBs on humans has been a concern for the past three decades. The goal of the present study was to investigate the effect of long-term exposure to PCBs on the development of dental enamel in children.

Materials and Methods

Study Population

In 1997, we enrolled 8- to 14-year-old children who were pre- and post-natally exposed to PCBs in the contaminated region of Bela Krajina, Slovenia. The study population was selected based on preliminary information, which included the data available from a national monitoring programme on levels of PCBs in human tissues and the environment, and a questionnaire completed by the parents. It contained items concerning child risk factors that can result in exposure to PCBs (e.g. place of residence, dietary habits).

Children had to meet the following criteria for inclusion: (1) lifelong residents in areas contaminated with PCBs, and eating locally grown food (dairy products, meat and fish), and/or (2) showing evidence of high levels of PCBs in maternal serum and/or breast milk (>0.7 mg/kg lipid). 202 children comprising the study group and 202 controls from Brsljin (about 30 km away) were matched for age (within 6 months) and sex. All parents gave informed consent.

The levels of PCBs in dentine of teeth from the study children were used to validate exposure. They had been extracted mainly for orthodontic and dental caries reasons. A total of 68 permanent teeth were obtained: 36 teeth were from the study group children and 32 from the control children.

Fluoride concentrations in drinking water collected in March and September 1997 were determined with an ion-selective electrode (model EA 940, Orion, Beverly, Mass., USA). Fluoride supplementation was the same in both study populations.

Determination of PCBs

The sample extracts (food, serum, milk) were analysed for PCBs by high-resolution gas chromatography using electron capture detection, as presented previously [Zupančič-Kralj et al., 1992]. Total PCB levels in food and dentine were calculated from the sum of the prevailing PCB congeners (IUPAC No. 28, 66, 101, 105, 118, 138, 153, 180) [Jan and Adamič, 1991]. In order to assess the potential toxicity of dioxin-like PCBs (IUPAC No. 105, 118, 126, 156, 157, 170) in food, the toxic equivalency factor approach was used [Hong et al., 1993; Safe, 1994; Giesy and Kannan, 1998]. The separation of planar (toxic) from non-planar PCB congeners was performed on a carbon cartridge [Zupančič-Kralj et al., 1991].

Analysis of PCB Levels in Dentine

Enamel and cementum were removed from dentine with a diamond burr. A pooled dentine sample from each area was ground and homogenized. 2–4 g of the dentine sample was ultrasonically treated with concentrated sulphuric acid and hexane. The combined hexane extracts were cleaned up with concentrated sulphuric acid and by saponification with KOH in ethanol [Smedes and de Boer, 1997], and purified by chromatography on micro-columns of silica and Florisil. The eluate was then analysed by high-resolution gas chromatography with electron capture detection. The values represent the arithmetic mean of three successive measurements.

Dental Examination

Dental examinations were carried out in 1997 by two calibrated dentists, operating blind, under natural light (bright sunlight avoided) using a standard mouth mirror and dental probe, as proposed by Clarkson and O'Mullane [1989]. The teeth were not cleaned or dried prior to examination. The epidemiological version of the modified Developmental Defects of Enamel (DDE) Index [FDI, 1992] was used on the recommended permanent (maxillary first premolars, canines and incisor teeth and mandibular first molars) and deciduous (maxillary incisors and canines and mandibular first molars) index teeth for collecting data relating to defects. This system records three main types of defect: demarcated opacities, diffuse opacities, and hypoplasia. Combinations of defects were recorded using additional codes. The extent of the defects was recorded in thirds of the surface area.

Ten percent of the study children were re-examined, and an analysis of the inter-examiner reproducibility was carried out according to Landis and Koch [1977], and intra-examiner reproducibility according to Shaw and Murray [1975].

All the data were analysed using the SPSS 6.0 statistical software package.

Table 1. Percentage of PCB-exposed children and of controls with at least one permanent tooth affected and percentage of teeth affected by different types of enamel defects on labial surfaces of permanent index teeth

Type of defect	Exposed		Control	
	mouth	teeth	mouth	teeth
Demarcated opacity	60.3	13.2	33.1	6.1
Diffuse opacity	29.1	6.5	30.6	7.0
Hypoplasia	20.7	3.8	4.9	0.6
Any defect	71.3	21.9	49.5[a]	12.7[b]

[a] $\chi^2 = 10.21$, $p = 0.0019$.
[b] $\chi^2 = 84.18$, $p = 0.0001$.

Results

The study group comprised a total of 202 children (95 girls, 107 boys, mean age 11.3 years, range 8–15 years). The control group also consisted of 202 children, matched for age and sex.

Risk assessment, expressed as the mean daily intake of toxic equivalents (TEQ) in exposed and control children was estimated to be 39 and 1–2 pg TEQ/kg body weight/day, respectively. A total of 68 permanent teeth (32 from exposed and 36 teeth from control children) were analysed for PCB residues to validate exposure. The mean levels of PCBs in dentine of exposed and control children were 38 and 7 ng/g, respectively. The mean concentration of fluoride in the water supply of both areas was low (<0.1 mg F/l).

The inter-examiner reliability kappa statistics of the two examiners was 0.91. The intra-examiner reproducibility analysis gave figures that were highest for hypoplasia (96.0%) and lowest for diffuse opacities (87.5%).

The percentages of children with at least one permanent index tooth affected and of permanent index teeth affected were significantly higher in the exposed group (table 1). The difference was mostly due to demarcated opacities and hypoplasia. When excluding diffuse opacities from the final statistical analysis, there was a significantly higher percentage of children affected in the exposed group ($\chi^2 = 4.74$; $p = 0.029$), and of teeth affected by demarcated opacities and/or hypoplasia ($\chi^2 = 8.17$; $p = 0.004$). The extent of the defects was also greater in the exposed group ($\chi^2 = 61.3$; $p = 0.0001$). In the exposed group, 19.2% of the affected teeth had one or more defects greater in size than one third the area (>1/3, ≤2/3) of the labial surface of the tooth, and 4.6% of the teeth had defects larger than two thirds (combination of defects). In the control group, 9.4% of the affected teeth had defects greater than one third, and none greater than two thirds. The teeth most frequently affected in the exposed group were incisors and premolars. No differences in the distribution of defects in pairs of homologous teeth were observed ($p = 0.4$). To determine the severity of enamel defects among the groups, the mean number of affected teeth per child was calculated; it was higher ($z = -3.9$; $p = 0.0001$) in the exposed group (mean = 1.72, SD = 1.57) than in the control group (mean = 0.95, SD = 1.35). No significant associations were found between PCB exposure and the percentage of children with at least one deciduous tooth affected ($\chi^2 = 2.05$; $p = 0.152$).

Discussion

Our results show that long-term exposure to PCB pollution is significantly associated with developmental defects of enamel.

The prevalences of various types of defects in the control group were similar to those observed in other studies from low-fluoride areas [Clarkson and O'Mullane, 1989; Downer et al., 1994; Ellwood and O'Mullane, 1994]. The difference in the prevalence of defects between the groups was mostly due to demarcated opacities and hypoplasia. The observation of induced enamel hypoplasia in exposed children is in accordance with studies on PCB-treated rats and non-human primates [Hashiguchi et al., 1985; McNulty, 1985], where selective toxic effects on ameloblasts and cells of stratum intermedium in the secretory stage of enamel development were reported. Hara [1985] found that an increased frequency of mottled enamel was reported in children born to occupationally exposed mothers, while contamination of PCBs with dioxins and furans cannot be definitely excluded. The proportion of children/teeth with diffuse opacities that are likely to be of fluoride etiology [Clarkson and O'Mullane, 1989] was similar in both study groups.

The distribution of defects was balanced within the dentition and many children with such defects had early- and late-forming teeth affected, suggesting that these defects resulted from a systemic factor acting over a long period of time. In a recent study, Patandin et al. [1999] compared proportions of cumulated PCB/dioxin intake during breast-feeding and long-term dietary exposure during different age periods. Since children in Bela Krajina were exposed lifelong to PCBs through the food chain, the dietary intake of PCBs had a larger effect on their total body burden than the exposure in utero. This is in accordance with our findings that primary teeth were not affected significantly, nor did the first permanent molars prove to be more sensitive to

polyhalogenated aromatic hydrocarbons, as was suggested [Brook et al., 1997] from the results of the study of Alaluusua et al. [1996b]. In this study, permanent first molars were chosen as target teeth so as to indicate lactational exposure to prevailing levels of PCDDs/Fs and PCBs.

Developmental defects of enamel in our study occurred at an estimated daily intake of TEQ only 4 times higher than the daily intake of 10 pg TEQ/kg body weight/day currently recommended tolerable by the WHO [Masuda, 1996], and are thus a very sensitive marker of PCB toxicity in humans.

The exact pathogenetic mechanism explaining how organochlorines cause enamel defects is not clearly understood. Changes in enzyme levels, hormones, growth factors, and their receptors are the principal known biochemical consequences of exposure to dioxin-like coplanar PCBs [Birnbaum, 1994]. Similarly, toxicity of TCDDs in cultured embryonic mouse teeth was shown to involve epidermal growth factor receptor signalling [Partanen et al., 1998]. However, non-coplanar PCBs in the mixture are likely to act through a different mechanism of action and may influence final toxicity outcomes [Giesy and Kannan, 1998].

In conclusion, our results show that long-term exposure to PCBs alone may cause developmental defects of enamel. Since for inhabitants of developed countries the majority of food TEQ is contributed by PCBs [Masuda, 1996], the possibility that it may cause developmental defects is a public health concern. Further evaluation of the mechanism of this toxicity is needed.

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